

# The IL-15-based superagonist ALT-803 promotes the antigen-independent conversion of memory CD8<sup>+</sup> T cells into innate-like effector cells with antitumor activity

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**Abbreviations:** IFN, interferon; IL, interleukin; IL-2R $\beta$ , IL-2 receptor  $\beta$ ; Lm, *Listeria monocytogenes*; NK, natural killer; OVA, ovalbumin; TAA, tumor-associated antigen; TCR, T-cell receptor

ALT-803, an interleukin-15-based superagonist, induces memory CD8<sup>+</sup> T cells to proliferate, upregulate receptors involved in innate immunity, secrete interferon  $\gamma$  and acquire the ability to kill malignant cells in the absence of antigenic stimulation. Thus, ALT-803 can promote the expansion and activation of memory CD8<sup>+</sup> T cells while converting them into innate immune effector cells that exhibit robust antineoplastic activity.

Harnessing cytotoxic CD8<sup>+</sup> T cells against neoplastic lesions has been a major goal of anticancer immunotherapy.<sup>1</sup> The clonal expansion and activation of these cells are triggered by antigen-specific interactions between their T-cell receptors (TCRs) and the cognate tumor-associated antigen (TAA) displayed in complex with MHC molecules on the surface of malignant or antigen-presenting cells.<sup>2</sup> Surprisingly, a recent report has shown that cytokine-based systemic immunotherapy can trigger the antitumor functions of memory CD8<sup>+</sup> T cells in the absence of specific antigenic stimulation.<sup>3</sup> In particular, following the co-administration of interleukin (IL)-2 and a CD40-targeting agonist antibody to mice, memory CD8<sup>+</sup> T cells underwent a rapid expansion, upregulated killer cell lectin-like receptor subfamily K, member 1 (KLRK1, best known as NKG2D) and granzyme B, and acquired broad lytic functions. These cells failed to upregulate programmed cell death 1 (PDCD1, best known PD-1)

and CD25, suggesting that their activation was independent of TCR signaling. Furthermore, the authors demonstrated that antigen specificity is not mandatory for the expansion and antitumor activity of memory CD8<sup>+</sup> T cells as elicited by systemic immunotherapy in TCR-transgenic mice. Immunotherapy-activated ovalbumin (OVA)-specific memory CD8<sup>+</sup> T cells were indeed capable of lysing OVA<sup>+</sup> as well as OVA<sup>-</sup> tumors in vitro and also mediated significant antineoplastic effects in vivo. Taken together, these findings indicate that memory CD8<sup>+</sup> T cells activated by IL-2 and CD40 signaling can acquire an unusual innate-like phenotype and become capable of mounting antigen-independent cytotoxic responses against tumor cells. Human T cells with a similar phenotype were observed in melanoma patients upon localized imiquimod-based immunotherapy,<sup>3</sup> suggesting that such immune responses may be conserved across species.

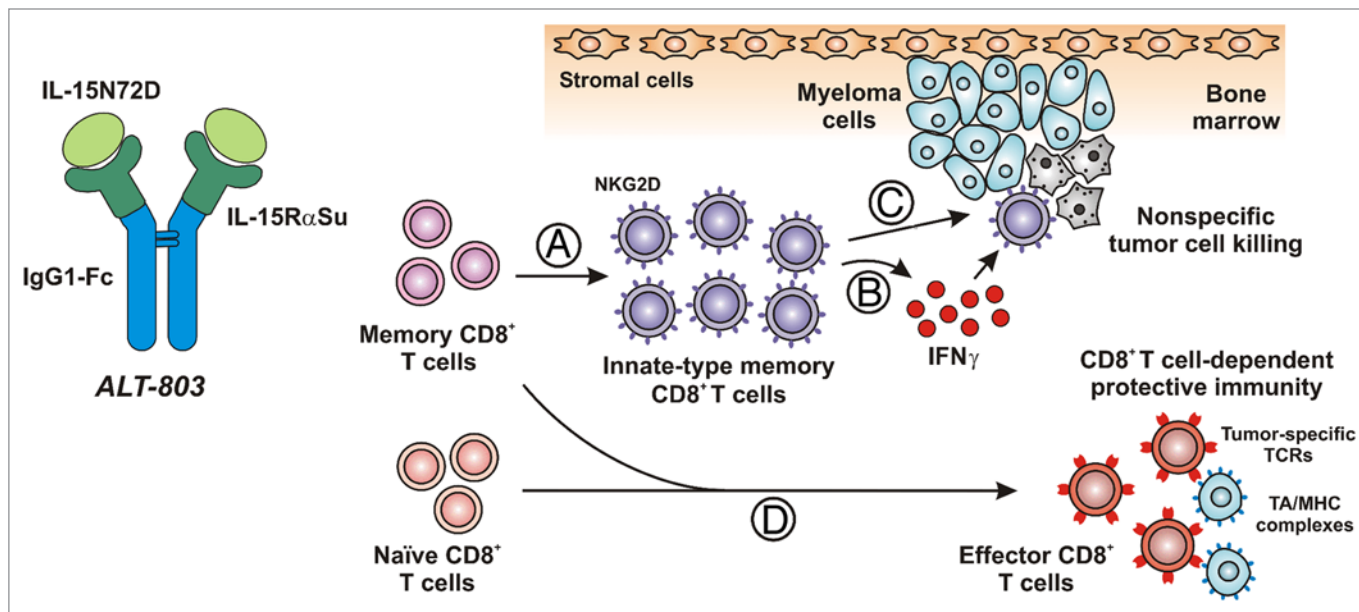
Recent studies have demonstrated that bacterial, viral and parasitic infections can

also trigger memory CD8<sup>+</sup> T cells to proliferate and become potent effector cells in the absence of specific antigenic stimulation via a process of natural inflammation known as “bystander” activation.<sup>4–6</sup> In a *Listeria monocytogenes* (Lm) immunization mouse model, Soudja et al. showed that Lm-specific memory CD8<sup>+</sup> T cells can acquire strong effector functions and expression of activation markers without the requirement for antigen recognition.<sup>4</sup> Such activation and differentiation of memory CD8<sup>+</sup> T cells into potent effector cells, which contribute to anti-bacterial immunity, was shown to be orchestrated by IL-15 and IL-18, which are secreted by inflammatory monocytes upon exposure to various classes of microbial pathogens. Along similar lines, Chu et al. subsequently showed that bystander-activated memory CD8<sup>+</sup> T cells can control the early pathogen load by killing target cells through an NKG2D-dependent mechanism, importantly mediating anti-influenza responses prior to the initiation of

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**Figure 1.** ALT-803 promotes innate-like CD8<sup>+</sup> T-cell effector activity and protective antitumor immunity in myeloma-bearing mice. ALT-803 is a supra-molecular complex that exhibits superagonist activity and is comprised of a mutant form of interleukin-15 (IL-15N72D) associated with a dimeric IL-15 receptor  $\alpha$  chain sushi domain (IL-15R $\alpha$ Su)–IgG1 Fc fusion. The N72D substitution confers to IL-15 increased affinity for the IL-2 receptor  $\beta$  chain (IL-2R $\beta$ ) and enhanced biological activity. In addition, association of IL-15N72D with IL-15R $\alpha$ Su further improves the biological activity of IL-15 in vivo, resulting in the potent activation of IL-2R $\beta$ / $\gamma$ -bearing natural killer (NK) cells and T lymphocytes. (A) In myeloma-bearing mice, ALT-803 promoted the rapid expansion of memory CD8<sup>+</sup> T cells but not naïve CD8<sup>+</sup> T lymphocytes. (B) Such memory CD8<sup>+</sup> T cells secreted high levels of interferon  $\gamma$  (IFN $\gamma$ ) and upregulated killer cell lectin-like receptor subfamily K, member 1 (KLRK1, best known as NKG2D) but not of programmed cell death 1 (PD-1) and CD25, on their surfaces. (C) ALT-803-activated cells also mediated nonspecific cytotoxicity against myeloma cells and other tumor cells, via a mechanism that was partially dependent on IFN $\gamma$ . By activating such a response, ALT-803 was capable of eliminating well-established myelomas from the bone marrow and significantly prolonging the survival of tumor-bearing mice. (D) Short-term ALT-803 treatment also provided tumor-bearing mice with protective immunity against a subsequent inoculation of myeloma cells. This protective response appeared to rely on CD8<sup>+</sup> T lymphocytes. Presumably, ALT-803 treatment stimulated naïve and/or memory CD8<sup>+</sup> T cells specific for tumor-associated antigens (TAAs) to acquire effector functions against a subsequent tumor challenge.

adaptive immunity.<sup>5</sup> In a mouse influenza model, Tietze et al. found that adoptively transferred OVA-specific memory CD8<sup>+</sup> T cells proliferated in the lungs and displayed increased levels of NKG2D, but not CD25, in response to influenza infection.<sup>6</sup> In this setting, the intranasal blockade of NKG2D resulted in a significant increase in viral replication in the early phase of infection. These studies demonstrate that microbial pathogens induce a rapid, antigen-independent expansion of memory CD8<sup>+</sup> T cells at the site of inflammation, resulting in the elicitation of NKG2D-dependent innate immune responses against infectious agents.

In studies described above, either multiple immunostimulatory proteins or inflammatory mediators were required to expand and activate memory CD8<sup>+</sup> T cells in the absence of specific antigenic stimulation. Conversely, we have recently shown that the systemic administration of

an IL-15 superagonist complex, ALT-803 (Fig. 1), is sufficient to trigger memory CD8<sup>+</sup> T-cell responses that mediate robust antitumor effects in several mouse models of myeloma.<sup>7</sup> ALT-803 contains a mutant form of IL-15 (IL-15N72D) that exhibits a 4–5-fold increase in biological activity as compared with wild-type IL-15 due to an improved affinity for the IL-2 receptor  $\beta$  chain.<sup>8</sup> In ALT-803, IL-15N72D is bound to a dimeric IL-15 receptor  $\alpha$  chain-IgG Fc fusion to form a complex with optimized in vivo activity, being at least 25-fold more potent than soluble IL-15.<sup>8,9</sup> In addition, ALT-803 has a significantly longer serum half-life in vivo than wild-type IL-15 (25 h vs. < 40 min). Hence, a single intravenous injection of ALT-803 is capable of inducing mouse CD8<sup>+</sup> T-cell and natural killer (NK)-cell proliferation at a dose at which an equimolar dose of IL-15 has no effects.<sup>9</sup> The administration of ALT-803 promoted the rapid expansion of memory

CD8<sup>+</sup> T cells, but not naïve CD8<sup>+</sup> T lymphocytes, in mice (Fig. 1).<sup>7</sup> Similar to what was reported in the context of the IL-2- and CD40-based immunotherapy,<sup>3,6</sup> ALT-803-activated memory CD8<sup>+</sup> T cells upregulated NKG2D, but not PD-1 or CD25, on their cell surface and secreted high amounts of interferon  $\gamma$  (IFN $\gamma$ ) without requiring antigen-specific stimulation in vivo. These lymphocytes also exhibited nonspecific cytotoxicity against cancer cells of several types, including myeloma cells, in vitro. Consistent with the results obtained with IL-2 and CD40-targeting agonist antibodies,<sup>3,10</sup> we found that these nonspecific memory CD8<sup>+</sup> T-cell responses could not be induced by IL-15 alone, indicating that the long-lived, potent immunostimulatory properties of ALT-803 might alleviate the requirement for CD40 (or other co-stimulatory) signaling.

The treatment of mice bearing 5T33 or MOPC-315 myelomas with ALT-803,

but not IL-15, rapidly eliminated malignant cells from the bone marrow and prolonged survival, often curing mice, in a CD8<sup>+</sup> T-cell dependent manner.<sup>7</sup> NK cells were not required for such anti-myeloma activity. Conversely, the ALT-803-mediated elevation of CD8<sup>+</sup> T cells in the bone marrow correlated with therapeutic responses, supporting the hypothesis that ALT-803 induces innate-like memory CD8<sup>+</sup> T cells that efficiently kill myeloma cells. Furthermore, as it also activates NK cells in vitro and in

vivo,<sup>9</sup> ALT-803 might have the potential to elicit broad innate immune responses against neoplastic and infected cells.

Finally, we observed that the curative, short-term administration of ALT-803 to tumor-bearing mice provided them with a CD8<sup>+</sup> T cell-dependent protection against a subsequent rechallenge with myeloma performed months later.<sup>7</sup> These findings suggest that ALT-803 also elicits efficient adaptive immune responses, resulting in the generation of long-term T cell-based antitumor immunity. Thus,

ALT-803 stands out as a potent immunostimulant that is capable of simultaneously activating the innate and adaptive arms of the immune system to elicit both rapid and long-lasting protective responses against infectious or neoplastic challenges to the host.

#### Disclosure of Potential Conflicts of Interest

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